Marine methane paradox explained by bacterial degradation of dissolved organic matter

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<table>
<thead>
<tr>
<th>Observed mass (Da)</th>
<th>Elemental formula (M+1)</th>
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<tr>
<td>82.9898</td>
<td>H₄O₃P</td>
<td>phosphite</td>
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Table S1. Phosphonate ions (M+1) observed in the high resolution mass spectrum of NELHA 2015 semi-labile DOM hydrolysis products. Phosphonates were extracted from the hydrolysis product with acidic methanol and further purified by ion exchange high pressure liquid chromatography. Phthalic anhydride (m/z 149.02383) was used as an internal calibrant.
Table S2. Aerobic growth of *P. stutzeri* HI00D01 during semi-labile DOM degradation.

Oxygen/argon (O₂/Ar) ratios obtained by membrane-inlet mass spectrometry were used to determine the initial and final dissolved oxygen (O₂) concentration (equation 1) in samples of *P. stutzeri* HI00D01 wild type and mutant 14-E11 (*phnK*491::Tn5) cultures grown in MOPS media amended with semi-labile DOM (95 mg L⁻¹) or methylphosphonate (1 µM). Control samples consisted of cultures grown in MOPS media with no additional phosphorus source. All samples contained glucose to a final concentration of 100 µM C and excess inorganic nitrogen in the form of ammonia (9.5 mM N). Initial and final cell concentrations (cells mL⁻¹) for each sample were determined by flow cytometry analysis to assess culture growth and viability.
Figure S1. $^{31}$P NMR and HMBC of semi-labile DOM. HMBC spectrum of (A) hydroxymethylphosphonate at 22.5 ppm x 3.70 ppm ($^{31}$P x $^1$H; $J_{PH} = 7$ Hz) (B) hydroxypropyl phosphonate (CH$_3$CH(OH)CH$_2$P-) at 22.8 ppm x 2.02 ppm ($J_{PH} = $ Hz), (C) phosphonoacetic acid (HOOCCH$_2$P-) at 16.89 ppm x 2.86 ppm ($J_{PH} = 21.1$ Hz; additional 21.3 Hz coupled doublet at 16.48 ppm x 2.88 ppm is from unhydrolyzed ester), and (D) phosphite at 4.68 ppm x 6.8 ppm ($J_{PH} = 672$ Hz). 1D projections of the summed $^{31}$P and $^1$H spectra appear next to the horizontal and vertical axes respectively.
Figure S2. Experiment 2: Evolution of methane (A), ethylene (B), oxygen (C) and total cell counts (D) during microbial degradation of semi-labile DOM sample NELHA 2015. Seawater was amended with semi-labile DOM to a final concentration of 49.7 mg L\(^{-1}\) (blue circles) compared to the control (white circles). Both treatments were amended with glucose and nitrate to a final concentration of 100 µM C and 16 µM N. Error bars represent one standard deviation of duplicate samples.
Figure S3. $^1$HNMR and UV Vis spectra of semi-labile DOM sample NELHA 2015. (A) The proton NMR spectra shows major signals at 4.5-5.5 ppm (anomeric H; (O)$_2$CH), 3.5-4.5 ppm (O-alkyl; HO-CH), 2.7 ppm (methyl amino sugar; CH$_3$N-), 2.0 ppm (acetamide; N-CO-CH$_3$), and 1.3 ppm (deoxysugar; HOC-CH$_3$) from the presence of polysaccharides in the sample. The peak at 4.7 ppm (*) is from the solvent (water). The spectrum is similar to $^1$HNMR spectra of surface and deep water DOM collected by ultrafiltration at other sites. (B) The UV/Visible spectrum between 220 and 350 nm showing a smooth decrease in adsorption with increasing wavelength characteristic of marine humic substances. Although we observe no distinct peak for nucleic acids at 260 nm (arrow) we use this wavelength to estimate the maximum possible contribution of nucleic acids to phosphorus in our samples.
Figure S4. *Pseudomonas stutzeri* HI00D01 phosphonate utilization growth tests.

Cultures of *P. stutzeri* HI00D01 wild type (WT) and mutant (MUT) strain 14-E11 (*phnK*491::Tn5) were spotted (10 µL) in duplicate on MOPS-based agarose media containing 0.2% glucose and inorganic phosphate (100 mM K$_2$HPO$_4$) or phosphonates (100 mM) as sole phosphorus source. Positive growth appears as circular, tan colored biofilm. Negative growth is indicated by absent or poor biofilm formation. *(A, left)* Growth test with 2-aminoethyl phosphonate (upper panel) and inorganic phosphate control (lower panel). *(B, right)* Growth test with methyl phosphonate (upper panel) and inorganic phosphate control (lower panel). In both tests, the strain *phnK*491::Tn5 failed to grow in media containing phosphonates as sole phosphorus source, but could readily achieve growth as the wild type strain when inorganic phosphate was supplied.